

Committee¹¹ is also participating actively in this initiative. The first ENMARIA workshop took place in conjunction with Resistance '97 at Rothamsted in April 1997, when plans for sharing data and experiences, compiling a European resistance database, and organising further meetings and training visits over the next 30 months were discussed and implemented. ENMARIA will facilitate the dissemination of results of nationally funded programmes as widely and rapidly as possible across Europe. Involvement in ENMARIA is open to all interested individuals and organisations; further details are available from the main coordinators whose details are listed below:

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Development and Implementation of Biochemical Insecticide Resistance Detection in Danish Field Strains of *Musca domestica*

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This summary discusses a project, the overall objectives of which are to identify the main resistance mechanisms, to develop and implement biochemical methods for studying them and to implement resistance management strategies for the most important agricultural pests in Denmark. In the initial phase of the project we have focused on insecticide resistance in houseflies caused by metabolism by general or specific esterases, by glutathione-S-transferase (GST), or by P450 monooxygenases, and by alteration of the organophosphate target acetylcholinesterase (AChE).

Monitoring of resistance in Danish field populations of houseflies (*Musca domestica* L.) related to the use of insecticides for fly control has been performed yearly since 1948.¹ Field-collected strains with different resistance patterns have, during this time, been isolated and are regularly selected. In this summary, we compare three *M. domestica* laboratory strains, the susceptible WHO, the multi-resistant 381zb and the azamethiphos-resistant 594vb, which were tested using biological and biochemical assays. We measured the enzymatic activity towards four naphthyl ester and three *p*-nitrophenyl ester substrates, GST activities with 1-chloro-2,4-dinitrobenzene (CDNB) and 3,4-dichloronitrobenzene (DCNB), monooxygenase activity with *p*-nitroanisole (PNA) and inhibition of AChE by azamethiphos and methomyl.

With dimethoate the 381zb strain has an R/S ratio of 59 at LD₅₀ and 56 at LD₉₅, which is synergised by neither DNF nor PBO. AChE activity of 381zb is less sensitive to inhibition by azamethiphos and methomyl

compared with susceptible flies and has similar sensitivity to that of the well-characterised 49r2b dimethoate-resistant strain, which has altered AChE activity.²

For permethrin, the 381bz strain has R/S of 615 at LD₅₀ and 773 at LD₉₅, which can be partly synergized by PBO but only slightly synergised by DEF. This strain has a super-*kdr* allele of the Na-channel protein gene^{3,4} and the pyrethroid resistance could be caused by a combination of the insensitive target site and metabolism due to GSTs and monooxygenases. The GST activity of the 381zb strain is significantly different from that of the susceptible WHO strain: DCNB activity is increased three-fold and CDNB activity is slightly elevated. We have not been able to link permethrin degradation directly to GST activity since it was neither an inducer nor an inhibitor of GST activity. The PNA monooxygenase activity of 381zb is *c.* two-fold greater than that of the WHO strain.

With the 594vb strain, the R/S with azamethiphos is 33 at LD₅₀ and 370 at LD₉₅; at LD₅₀ it is synergised by both PBO and DEF to R/S of 6 and 9, respectively. At the LD₉₅ the R/S level changes to 2 and 14 for PBO and DEF, respectively. The reaction rate of uninhibited AChE in 594vb is very high compared to the three other strains tested, but it is strongly inhibited by azamethiphos. Involvement of AChE in azamethiphos resistance in the 594vb strain has yet to be demonstrated. The GST and PNA monooxygenase activities of the 594vb and WHO strains are similar.

Alterations of general esterase level of substrate specificity in relation to insecticide resistance have not been identified previously in Denmark, although they have been studied in at least 12 field-collected Danish strains.⁵ We have measured esterase activity of the 381zb, 594vb and WHO strains using seven different esterase substrates (*p*-nitrophenylacetate, -propionate and -butyrate; α -naphthylacetate and -butyrate and β -naphthylacetate and -butyrate). There are subtle differences between males and females using some of the substrates and there are differences in the specific activity level with the different substrates. However, there is a high degree of correlation between the activity obtained with different esterase substrates, as shown by pairwise linear regressions. Thus, for screening of general elevated esterase activity, a single substrate will be sufficient.

The multiresistant 381zb strain contains many mechanisms which contribute to the observed resistance, like the well-characterised pyrethroid-resistant LPR strain where a combination of constitutively elevated P450 monooxygenase activity, insensitive target site and delayed penetration is responsible for the high level of resistance.⁶ The 594vb strain, on the other hand, shows only weak indices of resistance with our current biochemical assay and needs to be bioassayed to detect the resistance.

This collection of baseline data for susceptible and Danish field-collected strains of houseflies will explain the implementation of a survey strategy which combines biological and biochemical assays although, to get a complete picture of insecticide resistance in natural populations, we need to include molecular biology, determination of the Na-channel protein gene and GABA-receptor gene alleles.

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The Biochemical Detection of Insecticide Resistance in Danish Field Populations of the German Cockroach *Blattella germanica* (Blattellidae)

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Since the discontinuation of dieldrin use in the late 1970s due to resistance problems, the control of cockroaches in Denmark has relied on a strategy of alternate periods of chlorpyrifos/diazinon and permethrin/deltamethrin treatment. While resistance to pyrethroids arose within four years of their introduction, chlorpyrifos resistance sufficient to cause control problems has never been reported.¹ This is generally consistent with the experience in other parts of the world.